# Proceedings

## The 13th International Pig Veterinary Society Congress
## 26-30 June 1994, Bangkok, Thailand.

**Correction** page 505

5 % pentobarbital should read 0.5 % pentobarbital

line 2 in Abstract
line 10 in Materials and methods
line 1 in Table 1
line 2 in Table 2
line 7 in Discussion

Organized by Chulalongkorn University Faculty of Veterinary Science
Edited by Prachak Poomvises & Pringsri Ingkaninun

# PCR for identification of spirochaetes associated with intestinal spirochaetosis

N.Y. Park*[1], C.Y. Chung[1], A.J. McLaren[2], R.F. Atyeo[2] and D.J. Hampson[2]
1. College of Veterinary Medicine, Chonnam National University, Republic of Korea.
2. School of Veterinary Studies, Murdoch University, Western Australia.

## Introduction

Weakly haemolytic intestinal spirochaetes, distinct from both *Serpulina hyodysenteriae* and *Serpulina innocens*, have been reported to cause a disease called intestinal spirochaetosis, or spirochaetal diarrhoea, in weaned pigs (1, 2, 3, 4). Infected animals develop a sloppy mucoid diarrhoea and a reduction in growth rate. These spirochaetes are slightly more slender than *S. innocens*, and characteristically only have 4-6 axial flagellae (5). Strain P43/6/78, which was isolated from the first recorded case of porcine intestinal spirochaetosis (1), has been deposited with the American Type Culture Collection (as ATCC 51139) as the type strain of a possible new genus and species of spirochaete (*"Anguillina coli"*) (5). In the current study we have used a polymerase chain reaction (PCR) amplification of a section of the region encoding the 16s ribosomal RNA gene as a rapid and specific means of identifying these bacteria.

## Materials and methods

DNA was extracted from 74 intestinal spirochaetes, including 35 isolates of *Serpulina* spp. (*S. hyodysenteriae*, *S. innocens*, *"S. intermedius"* and *"S. murdochii"*) (5, 6), one each of *Brachyspira aalborgi* and *Treponema succinifaciens*, and 37 isolates of *"A. coli"* from pigs and humans. The spirochaetes were identified on morphological and biochemical criteria, and on their genetic grouping by multilocus enzyme electrophoresis (5, 6). The 16S sequence of these bacteria was amplified in PCR using a universal eubacterial 16S rRNA sequencing primer (1492r) and a 21 base primer designed to include a nucleotide sequence specific for *"A. coli"* (7). The annealing temperature was optimised to 48°C, with 35 cycles of amplification. The amplified product was subjected to electrophoresis in a 1.5% agarose gel and stained with ethidium bromide.

A series of dilutions of human *"A. coli"* strain HRM2 cells were made in 1ml sterile distilled water. These were then boiled for ten minutes, centrifuged, the supernatants discarded, and the pellets subjected to PCR using the same procedure described above.

## Results

As predicted, PCR product, approximately 1365 base pairs in length, was generated from the DNA of all porcine and human strains of *"A. coli"*. No product of this size was generated from the DNA of any of the other spirochaetes.

A PCR product of the same length was also generated from boiled cells of *"A. coli"* at dilutions down to $10^4$ cells per ml of water.

## Discussion

PCR amplification of a segment of the 16S rRNA gene was shown to be 100% sensitive and specific for identification of *"A. coli"* isolates of both porcine and human origin. Furthermore, the test was simple and rapid, and could be made more so by the use of boiled *"A. coli"* cells rather than extracted DNA. The limits of detection of the test in its current configuration, at $10^4$ cells per ml, was not particularly good, but might be improved by modifications such as using booster PCR. The test was not evaluated on faecal samples seeded with *"A. coli"* cells, but its use in this context could provide a rapid diagnostic technique for detection and identification of spirochaetes associated with intestinal spirochaetosis.

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## Acknowledgments

This study was supported by a grant from the Australian Pig Research and Development Corporation. NYP was in receipt of an International Reciprocal Fellowship with Korea, awarded by the Australian Research Council.